# Evaluation of Lectin-Expressing Transgenic Sugarcane Against Stalkborers (Lepidoptera: Pyralidae): Effects on Life History Parameters

M. SÉTAMOU, J. S. BERNAL,[1] J. C. LEGASPI,[2] T. E. MIRKOV,[2] AND B. C. LEGASPI, JR.[2]

Biological Control Laboratory, Texas A&M University, College Station, TX 77843–2475

J. Econ. Entomol. 95(2): 469–477 (2002)

**ABSTRACT** The impact of snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) expressed in transgenic sugarcane on life history parameters of Mexican rice borer [*Eoreuma loftini* (Dyar)] and sugarcane borer [*Diatraea saccharalis* (F.)] (both Lepidoptera: Pyralidae) was evaluated. In the laboratory, lyophilized sugarcane leaf sheath tissue was incorporated in a meridic diet resulting in a GNA concentration of 0.47% of total protein, and used for insect bioassays over two successive generations. Deleterious effects of GNA were not observed on survival, weight, and developmental periods of larvae and pupae, nor on adult fecundity and egg viability of *D. saccharalis.* Moreover, in the first generation, addition of transgenic sugarcane tissue to the diet enhanced larval growth in *D. saccharalis* resulting in higher larval and pupal weight compared with diet with nontransgenic sugarcane, but this effect was not observed in the second generation. In contrast, larval survival, percent adult emergence, and female fecundity of *E. loftini* were significantly reduced when fed transgenic sugarcane diet compared with nontransgenic sugarcane diet. In addition, a substantial reduction of female pupal weight of *E. loftini* was observed in the second generation. For both species, the only consistent effect of GNA in both generations was a reduction in adult female longevity. Life table parameters showed that GNA at the level found in the transgenic diet negatively affected development and reproduction of *E. loftini*, whereas it had a nil to positive effect on development and reproduction of *D. saccharalis.*

**KEY WORDS** *Eoreuma loftini*, *Diatraea saccharalis*, snowdrop lectin, *Galanthus nivalis* agglutinin, life history parameters

GENETICALLY ENGINEERED CROP cultivars resistant to insect pests are increasingly attracting interest as components of pest management strategies. All insect-resistant transgenic crops commercialized to date express toxins derived from the bacterium *Bacillus thuringiensis* Berliner (Schuler et al. 1998), but transgenic crops expressing plant-derived proteins, such as the snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) are now being evaluated. GNA is a lectin that specifically binds alpha-D-mannose (van Damme et al. 1998), and exhibits toxicity toward a number of insect pests belonging to different orders, including Homoptera (Powell et al. 1995), Coleoptera (Leple et al. 1995, Nutt et al. 1999), and Lepidoptera (Fitches et al. 1997, Gatehouse et al. 1997). Transgenic lines expressing GNA have been generated for several crop species including potato (Birch et al. 1999), tobacco (Hilder et al. 1987), wheat (Stoger et al. 1999), rice (Rao et al. 1998), and sugarcane (Allsopp and McGhie 1996, Irvine and Mirkov 1997). The effects of GNA consumption on insects may vary among species because of variability in insect feeding behavior and digestion. Hence, some species are highly susceptible to GNA, and the effects on others may be nil or moderate.

Transgenic sugarcane expressing GNA was developed at the Texas Agricultural Experiment Station (TAES) in Weslaco. A GNA-producing gene was introduced from snowdrop lily (*Galanthus nivalis*) into the sugarcane variety CP65–357 using maize ubiquitin as the promoter and the paint-sprayer delivery technique (Irvine and Mirkov 1997). Using this technique, lectin was expressed in both leaves and stalk storage tissue of sugarcane.

The objective of this study was to assess the effects of snowdrop lectin, GNA, on life history parameters and feeding damage of the Mexican rice borer [*Eoreuma loftini* (Dyar)] and sugarcane borer [*Diatraea saccharalis* (F.)] (both Lepidoptera: Pyralidae). *Diatraea saccharalis* and *E. loftini* are pests of sugarcane in the Lower Rio Grande Valley, TX. Currently, *D. saccharalis* is biologically controlled by *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) and is a secondary pest, whereas *E. loftini*

[1] E-mail: juliobernal@tamu.edu.
[2] Texas Agricultural Experiment Station, Weslaco, TX 78596–8399.

is the primary pest of sugarcane in the Lower Rio Grande Valley and no efficient control measure has been found (Pfannenstiel and Meagher 1991, Legaspi et al. 1997). Insight on the potential effects of widespread deployment of transgenic sugarcane on the population dynamics of both borer species may be gained by evaluating the effects on life history parameters of borer larvae feeding on a diet containing GNA or transgenic sugarcane.

## Materials and Methods

**Test Insects.** *Eoreuma loftini* and *D. saccharalis* were obtained from laboratory cultures at TAES, Weslaco. Both species were maintained on artificial diet (Martinez et al. 1988) at 25°C, 65% RH, and a photoperiod of 14:10 (L:D) h as described by Rodríguez-del-Bosque et al. (1989). Eggs newly laid on waxed paper or paper, the oviposition substrates, were incubated to larval emergence. Eggs of *D. saccharalis* on waxed paper were placed in sealed plastic bags, and incubated at 25 ± 2°C under a photoperiod of 12:12 (L:D) h for 8–10 d in glass jars containing tap water saturated with NaCl as a source of humidity. Eggs of *E. loftini* on paper strips were kept in glass vials and incubated under the same ambient conditions as those of *D. saccharalis.*

**Plant Material.** Transgenic line 83, expressing snowdrop lectin, and nontransgenic sugarcane were planted in fields at TAES, Weslaco. All plants were grown in blocks of 100 $m^2$. Nitrogen fertilizer was applied ≈2 wk after planting at a rate of 75 kg/ha, and plants were flood-irrigated at 2-wk intervals.

**Leaf Sheath Tissue and Diet Treatments.** Leaf sheaths were collected from ≈8-mo-old plants, sealed in plastic bags, and stored at –15°C in a deep freezer. Leaf sheath tissue was subsequently dried in a freeze-dryer (Duradry Condenser Module, FTS systems, Stone Ridge, NY) for 96 h. Dried tissue was ground with a laboratory mill (Thomas Scientific, Swedesboro, NJ) and sifted through a 40-mesh screen (Thomas Scientific). The resulting powder was sealed in plastic bags and stored in a deep-freezer between –20 and –15°C until used in diet preparation.

Three diets were used for the bioassays. The artificial diet (Martinez et al. 1988) used in the laboratory for rearing the borers was tested as the "control" diet. The two remaining diets consisted of the control diet with leaf sheath tissue added from either transgenic sugarcane or nontransgenic sugarcane, each at a concentration of 10 g of tissue per 150 g of total diet weight (Meagher et al. 1996); hereafter, these diets are referred to as "transgenic" or "nontransgenic," respectively. Sugarcane tissue was added to the diet at the last stage of preparation, when the diet temperature was <70°C to avoid affecting the stability of GNA (Kaku and Goldstein 1989). The diet was poured in small cups at a rate of ≈5 g per cup and allowed to cool to room temperature before use.

**Determination of Extractable Protein and Transgene Expression.** Before feeding to insects, the total amounts of soluble protein in the three diets, level of GNA expression in sugarcane tissues (transgenic and nontransgenic), and GNA concentration in each of the diets were determined using western blot and immunostaining procedures. Ground leaf sheath tissue and the different diets were homogenized in 1 × SDS extraction buffer containing 63 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, and 5% b-mercaptoethanol. Total protein concentration was estimated using the method described by Bradford (1976). The proteins were separated using SDS-page gels comprising a 15% resolving gel and a 4.5% stacking gel. Fifty microliters of each sample extract was loaded within the gel lanes, and electrophoresis was run at 100 V for 2 h. The protein bands were transferred to a nitrocellulose membrane, which was blocked overnight to saturate nonspecific protein binding sites. The membrane was thereafter transferred in a primary and secondary antibody binding solution containing goat anti-rabbit immunoglobulin (IgG). The protein bands on the membrane were stained, air-dried at room temperature and visualized.

**Artificial Diet Evaluation.** *Experimental Protocol.* For both *D. saccharalis* and *E. loftini*, single 1-d-old neonate larvae were placed in cups containing diet using a fine camel's-hair brush. The cups were covered with lids, placed in trays, and incubated at 30 ± 1°C, 70 ± 2% RH, and a photoperiod of 12:12 (L:D) h regime. Both *D. saccharalis* and *E. loftini* were reared for two successive generations under these conditions. Three treatments were used in the first generation: control, nontransgenic, and transgenic diets. Two treatments were used in the second generation: nontransgenic and transgenic diets. Four trays of 25 diet cups each, with 100 larvae of *D. saccharalis*, and three trays with 75 larvae of *E. loftini* were used per treatment in the first generation. Three trays of 20 diet cups each, with 60 larvae of each species of borer were used per treatment in the second generation.

Pupae were collected daily after the onset of pupation and immediately weighed and sexed. They were kept in sterile cups covered with a lid until adult emergence under the same ambient conditions as the larvae. Upon their emergence, adults from each treatment were mated separately by placing individual pairs (1 ♀ + 1 ♂) in clear plastic vials (9.5 cm high by 4.5 cm diameter). The lids of the vials were ventilated, and a piece of cotton wool soaked with 50% honey solution (vol:vol) was placed on top of each lid as a food source for the adults. The vials were held at conditions conducive to maximum fecundity and egg viability, 22 ± 2°C, 60 ± 5% (Rodríguez-del-Bosque et al. 1989). Waxed paper and paper strips for *D. saccharalis* and *E. loftini*, respectively, were placed inside the vials as oviposition substrates for the females. Groups of randomly selected eggs from each treatment were incubated at the same conditions as the adults.

*Data Collected.* Survival of larvae was recorded weekly for the first 3 wk after their placement in cups with diet. The percentages of pupae formed and adults emerged were determined. The weights of larvae at 3 wk and of newly formed pupae were measured

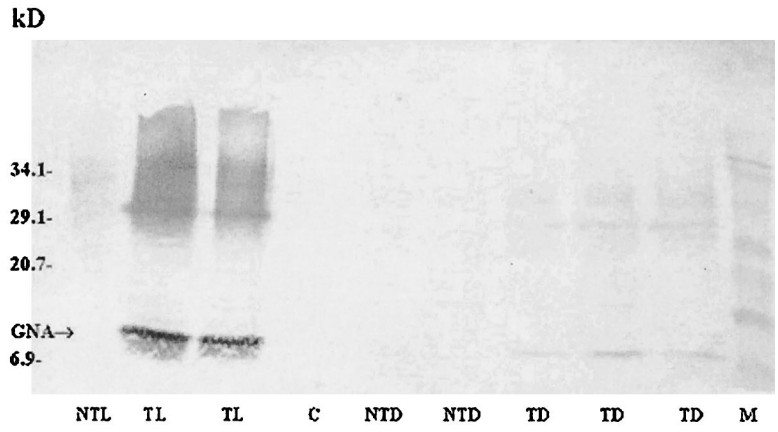


**Fig. 1.** Western blot analysis patterns of sugarcane leaf sheath tissue and different artificial diets used in the experiments. TL, transgenic leaf sheath; NTL, nontransgenic leaf sheath; C, control diet; TD, transgenic diet; NTD, nontransgenic diet; and M , marker. Protein molecular weight (kD) is indicated in the column on the left.

to the nearest 0.1 mg. The durations of the larval and pupal stages in days were recorded. All pupae were sexed, and the sex ratio (proportion ♂ ♂) was determined for each borer species and treatment. The longevity of adults (days) and fecundity (number of eggs laid) of females were recorded. Developmental times for each life stage (L) were computed as $L = n_i x_i / n_i$, where $n_i$ is the number of individuals and $x_i$ the time required to complete the developmental stage. The viability of eggs, calculated as the proportion of larvae hatching from incubated eggs, was recorded. A jackknife program was used to calculate life table statistics for both *D. saccharalis* and *E. loftini* on the different diets (Hulting et al. 1990). The "growth index," computed as the ratio between the mean percentage of adults emerged and the mean duration of the immature period (Sétamou et al. 1999) was calculated for one generation for individuals reared on the control diet, and over two generations for individuals reared on the nontransgenic and transgenic diets.

**Statistical Analysis.** Homogeneity of the larval survival curves on the different diets was tested by a likelihood ratio using the LIFETEST procedure of SAS (SAS Institute 1996). Analysis of variance (ANOVA) was performed to evaluate the effects of

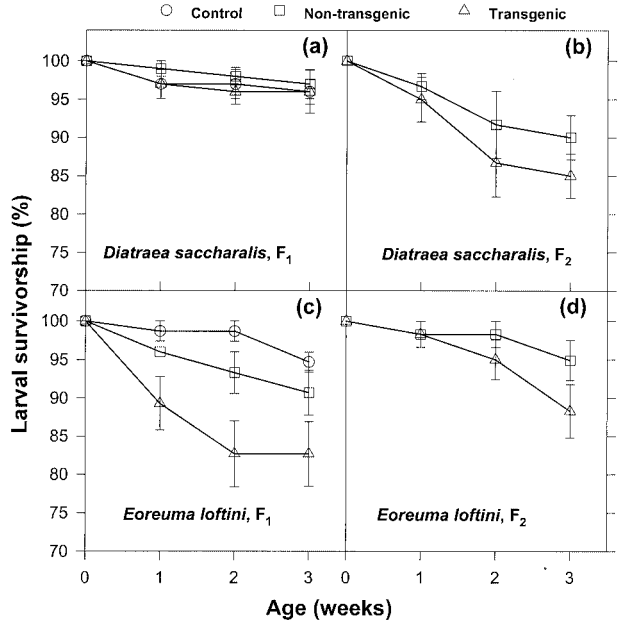


**Fig. 2.** Survivorship of larvae (±SE) of *Diatraea saccharalis* fed one of several diets during the (a) first and (b) second generations, and of *Eoreuma loftini* during the (c) first and (d) second generations.

treatments and generations on percent pupation and adult emergence, larval and pupal periods, larval and pupal weights, fecundity of females, viability of eggs, and life table parameters. Means were separated using the Tukey test when significant $F$ values were obtained ($P < 0.05$) (Sokal and Rohlf 1995). All percentages and proportions were arcsine$\sqrt{x}$-transformed before analysis, whereas results are presented as back-transformed data. Sex ratios were tested for conformity with a 0.5 sex ratio using the Wilcoxon chi-square test of conformity, and the log-likelihood ratio was used to test for homogeneity of sex ratios between diets (Zar 1996).

## Results

**Total Extractable Protein and Transgenic Expression.** Total extractable protein varied between 0.30 $\mu$g/$\mu$l in the control diet to 0.35 $\mu$g/$\mu$l in the transgenic diet, with an intermediate ratio of 0.32 $\mu$g/$\mu$l in the nontransgenic diet. The level of expression of GNA in the transgenic sugarcane leaf sheath tissue used in diet preparation was 0.89% of total extractable protein. The transgenic diet had a GNA concentration of 0.47% of total extractable protein, and GNA was absent in nontransgenic leaf sheath tissue, and control and nontransgenic diets (Fig. 1).

**Artificial Diet Evaluation.** *Diatraea saccharalis.* Survivorship of larvae of *D. saccharalis* did not vary significantly with diet in both generations (Fig. 2a and b). Consequently, significant differences were not observed in percent pupation and percent adult emergence of *D. saccharalis* on the various diets in both first and second generations (Fig. 3a). Moreover, significant generation effects on larval survival, and pupal and adult emergence rates were not evident for *D. saccharalis.*

Larvae of *D. saccharalis* fed transgenic diet had weight comparable to larvae fed control diet in the first generation, and weighed significantly more than larvae fed nontransgenic diet in both generations (Table 1). Both female and male pupae of *D. saccharalis* reared on the transgenic diet weighed more than pupae reared on the nontransgenic diet in the first generation but not in the second generation (Table 1).

The larval period of males of *D. saccharalis* was longer on nontransgenic relative to transgenic diet in the first but not second generation, whereas that of females was similar among diets in both generations (Table 2). Independent of diet, females of *D. saccharalis* had longer larval periods than males ($F = 85.96$;

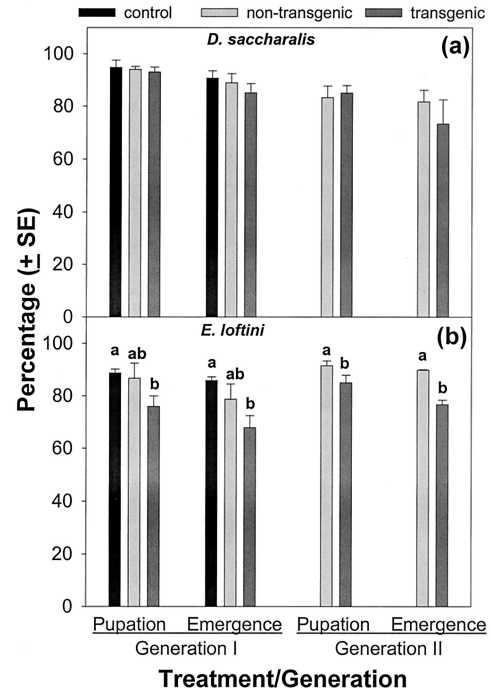


**Fig. 3.** Pupation and adult emergence rates of (a) *Diatraea saccharalis* fed one of several diets during the first and second generations, and (b) *Eoreuma loftini* during the first and second generations. Letters above columns indicate significant differences within groups of columns ($P < 0.05$); differences are not significant within groups of columns lacking letters ($P > 0.05$).

**Table 1. Mean weights (±SE) of larvae and pupae of *D. saccharalis* and *Eoreuma loftini* fed one of several diets over two consecutive generations**

| Generation | Diet | Larvae, mg | *N* | Pupae, mg | | | |
|---|---|---|---|---|---|---|---|
| | | | | ♂♂ | *n* | ♀♀ | *n* |
| | | | *Diatraea saccharalis* | | | | |
| $F_1$ | Control | 134.7 ± 6.12a | 71 | 94.9 ± 2.2a | 46 | 137.8 ± 3.1a | 47 |
| | Nontransgenic | 117.5 ± 3.28b | 95 | 80.1 ± 2.1c | 45 | 118.9 ± 2.7b | 48 |
| | Transgenic | 135.8 ± 4.79a | 74 | 88.6 ± 1.6b | 53 | 135.8 ± 2.6a | 39 |
| $F_2$ | Nontransgenic | 112.1 ± 5.21b | 46 | 82.9 ± 2.2a | 25 | 115.4 ± 4.9a | 25 |
| | Transgenic | 126.1 ± 6.20a | 41 | 85.2 ± 2.3a | 30 | 128.5 ± 5.9a | 21 |
| | | | *Eoreuma loftini* | | | | |
| $F_1$ | Control | 55.1 ± 4.8a | 68 | 44.2 ± 2.5a | 24 | 77.4 ± 2.7a | 38 |
| | Nontransgenic | 61.8 ± 3.6a | 65 | 36.1 ± 1.1b | 31 | 64.5 ± 2.3b | 34 |
| | Transgenic | 62.2 ± 4.5a | 61 | 33.6 ± 1.3b | 29 | 65.2 ± 1.8b | 27 |
| $F_2$ | Nontransgenic | 65.2 ± 4.0a | 54 | 37.3 ± 0.8a | 27 | 73.8 ± 2.1a | 27 |
| | Transgenic | 57.9 ± 3.1a | 54 | 35.8 ± 1.2a | 27 | 57.7 ± 3.0b | 24 |

Means followed by different letters within a column and generation are significantly different ($P < 0.05$, Tukey-test).

**Table 2. Length of developmental periods (±SE) and longevity of adults (±SE) of *Diatraea saccharalis* and *Eoreuma loftini* fed one of several diets over two consecutive generations**

| Generation | Diet | Larval period (days) | | Pupal period (days) | | Longevity (days) | |
|---|---|---|---|---|---|---|---|
| | | ♂♂ | ♀♀ | ♂♂ | ♀♀ | ♂♂ | ♀♀ |
| | | *Diatraea saccharalis* | | | | | |
| $F_1$ | Control | 22.0 ± 0.5ab | 25.3 ± 0.5a | 7.3 ± 0.2a | 6.7 ± 0.1a | 3.8 ± 0.2b | 3.8 ± 0.2b |
| | *n* | 27 | 47 | 46 | 43 | 46 | 43 |
| | Nontransgenic | 24.1 ± 0.4a | 26.1 ± 0.3a | 6.6 ± 0.1b | 6.4 ± 0.1a | 4.1 ± 0.2ab | 4.4 ± 0.2a |
| | *n* | 45 | 48 | 40 | 46 | 36 | 41 |
| | Transgenic | 21.7 ± 0.2b | 25.1 ± 0.4a | 6.9 ± 0.1b | 6.8 ± 0.1a | 4.5 ± 0.2a | 3.8 ± 0.2b |
| | *n* | 53 | 39 | 50 | 35 | 50 | 35 |
| $F_2$ | Nontransgenic | 21.9 ± 0.4a | 24.6 ± 0.7a | 6.8 ± 0.2a | 6.8 ± 0.3a | 4.5 ± 0.2a | 4.5 ± 0.3a |
| | *n* | 25 | 25 | 24 | 25 | 24 | 25 |
| | Transgenic | 22.1 ± 0.4a | 25.5 ± 0.7a | 6.5 ± 0.2a | 6.5 ± 0.2a | 4.1 ± 0.2b | 3.9 ± 0.2b |
| | *n* | 30 | 21 | 27 | 17 | 27 | 17 |
| | | *Eoreuma loftini* | | | | | |
| $F_1$ | Control | 29.2 ± 1.0a | 29.8 ± 0.7a | 8.2 ± 0.3a | 8.9 ± 0.2a | 8.7 ± 0.6a | 8.9 ± 0.4a |
| | *n* | 24 | 38 | 23 | 35 | 22 | 35 |
| | Nontransgenic | 29.3 ± 0.8a | 30.7 ± 0.7a | 8.2 ± 0.2a | 8.4 ± 0.2ab | 7.3 ± 0.4b | 7.7 ± 0.4b |
| | *n* | 31 | 34 | 26 | 33 | 21 | 28 |
| | Transgenic | 28.6 ± 0.8a | 29.2 ± 0.6a | 7.8 ± 0.3b | 7.9 ± 0.2b | 6.7 ± 0.5b | 7.1 ± 0.4b |
| | *n* | 29 | 27 | 25 | 25 | 19 | 16 |
| $F_2$ | Nontransgenic | 26.1 ± 0.5a | 29.8 ± 0.4a | 7.7 ± 0.1a | 7.2 ± 0.1a | 9.4 ± 0.4a | 8.9 ± 0.3a |
| | *n* | 27 | 27 | 26 | 27 | 26 | 27 |
| | Transgenic | 27.4 ± 0.7a | 30.0 ± 0.7a | 7.8 ± 0.1a | 7.5 ± 0.1a | 7.7 ± 0.4b | 6.3 ± 0.4b |
| | *n* | 27 | 24 | 24 | 22 | 22 | 22 |

Means followed by different letters within a column and generation are significantly different ($P < 0.05$, Tukey-test).

df = 1, 272; $P < 0.0001$ for the first generation, and $F = 32.90$; df = 1, 100; $P < 0.0001$ for the second generation; Table 2). The pupal periods of both sexes of *D. saccharalis* did not differ between transgenic and nontransgenic diets (Table 2).

The longevity of adult females was shorter on transgenic relative to nontransgenic diet in both generations. In contrast, longevity of males was shorter only in the second generation (Table 2). The sex ratio of *D. saccharalis* did not differ from a 1:1 ratio and did not vary with diet in each of two generations (Table 3). The fecundity and oviposition period of females, and the viability of eggs of *D. saccharalis* were not affected by diet in both generations (Table 4).

Life table parameters of *D. saccharalis* fed the control, transgenic, and nontransgenic diets are presented in Table 5. The net reproductive rate, intrinsic rate of increase and total progeny of *D. saccharalis* were highest on transgenic diet. In contrast, finite rates of increase, generation times, and growth indices were similar between diets, and doubling time appeared lowest on transgenic diet.

*Eoreuma loftini.* Survivorship of *E. loftini* varied significantly with diet in both generations (Fig. 2c and d). Pairwise comparison using the Z-statistic derived from the covariance matrix of the Wilcoxon statistic revealed that larval survivorship of *E. loftini* on transgenic diet was significantly lower after 3 wk than that on the control and nontransgenic diets in the first generation (Wilcoxon $\chi^2 = 6.54$, df = 2, $P = 0.038$), and nontransgenic diet in the second generation (Wilcoxon $\chi^2 = 3.95$, df = 1, $P = 0.043$). Significant differences were not evident between the control and nontransgenic diets in the first generation (Fig. 2c).

**Table 3. Sex ratio (proportion ♂♂) of *Diatraea saccharalis* and *Eoreuma loftini* fed one of several diets over two consecutive generations**

| Generation | Diet | Sex ratio ± SE | *P* that sex ratio = 0.5[a] | *n* |
|---|---|---|---|---|
| | | *Diatraea saccharalis* | | |
| $F_1$ | Control | 0.49 ± 0.05a | 0.92 | 93 |
| | Nontransgenic | 0.48 ± 0.05a | 0.78 | 93 |
| | Transgenic | 0.57 ± 0.05a | 0.20 | 93 |
| $F_2$ | Nontransgenic | 0.48 ± 0.07a | 0.80 | 50 |
| | Transgenic | 0.59 ± 0.07a | 0.22 | 51 |
| | | *Eoreuma loftini* | | |
| $F_1$ | Control | 0.38 ± 0.06a | 0.06 | 63 |
| | Nontransgenic | 0.48 ± 0.06a | 0.72 | 65 |
| | Transgenic | 0.53 ± 0.07a | 0.71 | 57 |
| $F_2$ | Nontransgenic | 0.50 ± 0.07a | 1.00 | 54 |
| | Transgenic | 0.52 ± 0.07a | 0.80 | 52 |

Values followed by same letter within column and generation are not significantly different ($P > 0.05$, log-likelihood ratio test).
[a] Wilcoxon chi-square test of conformity.

**Table 4. Fecundity of females (mean number of eggs laid (±SE), oviposition period (±SE), and viability of eggs (±SE) of *Diatraea saccharalis* and *Eoreuma loftini* fed one of several diets over two consecutive generations**

| Generation | Diet | Fecundity | *n* | Oviposition period (days) | *n* | Viability of eggs | *n* |
|---|---|---|---|---|---|---|---|
| | | | *Diatraea saccharalis* | | | | |
| $F_1$ | Control | 106 ± 10a | 21 | 8.8 ± 0.4a | 19 | 28.7 ± 3.0a | 5 |
| | Nontransgenic | 77 ± 11a | 19 | 9.3 ± 0.3a | 16 | 28.1 ± 1.9a | 5 |
| | Transgenic | 86 ± 12a | 26 | 9.5 ± 0.4a | 21 | 27.1 ± 2.3a | 5 |
| $F_2$ | Nontransgenic | 94 ± 21a | 18 | 8.9 ± 0.4a | 13 | 27.2 ± 2.8a | 5 |
| | Transgenic | 103 ± 17a | 13 | 9.3 ± 0.5a | 12 | 26.1 ± 3.0a | 5 |
| | | | *Eoreuma loftini* | | | | |
| $F_1$ | Control | 370 ± 27a | 17 | 8.8 ± 0.3a | 15 | 65.7 ± 8.3a | 6 |
| | Nontransgenic | 281 ± 17b | 20 | 9.2 ± 0.3a | 17 | 73.0 ± 8.0a | 7 |
| | Transgenic | 201 ± 25c | 19 | 9.2 ± 0.4a | 16 | 73.9 ± 9.1a | 7 |
| $F_2$ | Nontransgenic | 286 ± 39a | 13 | 8.8 ± 0.3b | 11 | 66.6 ± 6.0a | 7 |
| | Transgenic | 191 ± 33a | 13 | 9.9 ± 0.5a | 11 | 66.0 ± 5.9a | 6 |

Means followed by different letters within a column and generation are significantly different ($P < 0.05$, Tukey-test).

The percent pupation and adult emergence also varied significantly with diet in both generations of *E. loftini* (Fig. 3b). Significantly fewer pupae were formed and fewer adults emerged from transgenic diet relative to control diet in the first generation, and nontransgenic diet in the second generation. However, generation effects were not significant on larval survival, and pupal and adult emergence rates of *E. loftini.*

*Eoreuma loftini* larval weight did not differ with diet in both generations (Table 1). Male pupae of *E. loftini* had similar weights on transgenic and nontransgenic diets in both generations, while female pupae had similar weights in the first generation but lower weight on transgenic relative to nontransgenic diet in the second generation (Table 1).

The larval periods of both males and females of *E. loftini* did not differ with diet in both generations (Table 2). However, females had longer larval periods than males regardless of diet in the second generation ($F = 24.60$; df = 1, 101; $P < 0.0001$; Table 2). The pupal periods of both male and female *E. loftini* did not differ between transgenic and nontransgenic in the second generation. However, in the first generation, males of *E. loftini* had a slightly shorter pupal period on transgenic diet (Table 2).

The adult longevity of both *E. loftini* males and females on transgenic diet was shorter than that on nontransgenic diet in the second generation, whereas differences among these diets were not evident in the first generation (Table 2).

The sex ratios of *E. loftini* did not differ with diet in each of two generations (Table 3). These sex ratios tended to be unbiased, except in first generation *E. loftini* fed control diet (Table 3).

Larval diet significantly affected reproduction of *E. loftini.* The fecundity of females of *E. loftini* was significantly lower on transgenic relative to control and nontransgenic diets in the first generation, whereas it was similar between transgenic and nontransgenic diets in the second generation (Table 4). In addition, the oviposition period of females of *E. loftini* was longer in the transgenic relative to the nontransgenic diet in the second generation (Table 4). Differences were not evident in the viability of eggs of *E. loftini* in both generations (Table 4).

Life table parameters for *E. loftini* on control, transgenic, and nontransgenic diets are presented in Table 5. The net reproductive rate and total progeny were lowest on transgenic diet relative to nontransgenic and control diets. The intrinsic rate of increase was intermediate on nontransgenic diet, and was greater on transgenic versus control diet. In contrast, generation times and finite rates of increase were similar between diets, and doubling time appeared slightly

**Table 5. Life table parameters (±SE) and growth index (GI) of *Diatraea saccharalis* and *Eoreuma loftini* fed one of several diets**

| Diet | $R_0$ | *r* | λ | *T* | DT | Total progeny | GI |
|---|---|---|---|---|---|---|---|
| | | | *Diatraea saccharalis* | | | | |
| Control | 15.5 ± 1.4a | 0.066 ± 0.002a | 1.07 | 41.6 | 10.5 | 29.7 ± 2.6a | 2.95 |
| Nontransgenic | 12.0 ± 1.7a | 0.059 ± 0.006a | 1.06 | 42.0 | 11.7 | 23.3 ± 3.3a | 2.77 |
| Transgenic | 37.1 ± 4.6b | 0.089 ± 0.005b | 1.09 | 40.7 | 7.8 | 77.7 ± 9.6b | 2.64 |
| | | | *Eoreuma loftini* | | | | |
| Control | 122.1 ± 9.8a | 0.096 ± 0.002a | 1.10 | 50.2 | 7.2 | 196.9 ± 15.9a | 2.22 |
| Nontransgenic | 84.0 ± 5.3b | 0.088 ± 0.003ab | 1.09 | 50.3 | 7.9 | 164.2 ± 10.4a | 2.21 |
| Transgenic | 48.0 ± 4.9c | 0.079 ± 0.004b | 1.08 | 49.2 | 8.8 | 99.9 ± 10.1b | 1.94 |

$R_0$, net reproductive rate; r, intrinsic rate of increase; λ, finite rate of increase; T, generation time in days; DT, doubling time in days. Growth index, computed as ratio between percentage of adults emerged and mean duration of immature period for each diet (larval and pupal periods only) (Sétamou et al. 1999). Newmann-Keuls sequential test was used to compare life table parameters (Hulting et al. 1990).

greater in transgenic relative to nontransgenic and control diets.

## Discussion

The results of this study showed that feeding on transgenic diet containing ≈0.5% GNA substantially affected a number of life history parameters in *E. loftini.* Specifically, larval survivorship during each of the two generations, percent pupation, adult emergence rate, pupal weight, fecundity, and longevity in the second generation were lower in *E. loftini* fed transgenic diet relative to nontransgenic diet. However, pupal period was shorter and oviposition period longer in *E. loftini* fed transgenic relative to nontransgenic diet. Overall, the small differences in these parameters between *E. loftini* fed transgenic or nontransgenic diet translated into significant differences in the corresponding life table parameters. *Eoreuma loftini* fed transgenic diet had a lower net reproductive rate and intrinsic rate of increase relative to those fed nontransgenic or control diet. In contrast, the results showed that life history parameters were little to positively affected in *D. saccharalis* feeding on transgenic diet containing ≈0.5% GNA in the total extractable protein. Specifically, differences were not evident between *D. saccharalis* fed transgenic or nontransgenic diet in larval survivorship, pupation and adult emergence rates, female fecundity, oviposition period, and viability of eggs. Moreover, larval weight was greater in each of the two generations tested, and pupal weight was greater and larval period shorter in the first generation in *D. saccharalis* fed transgenic compared with nontransgenic diet. Longevity of females in both generations and males in the second generation was shorter in *D. saccharalis* fed transgenic relative to nontransgenic diet. The positive influences of the transgenic diet on *D. saccharalis,* i.e., greater larval and pupal weights and shorter larval period, resulted in favorable life table parameters associated with this diet. Net reproductive rates and intrinsic rates of increase were greater in *D. saccharalis* fed transgenic relative to nontransgenic diet.

GNA at the level occurring in the transgenic diet differentially affected development and reproduction of *D. saccharalis* and *E. loftini.* Overall, GNA substantially affected *E. loftini*, while except for a negative influence on longevity, it appeared to have no effect or even favor development and reproduction of *D. saccharalis.* These results suggest that GNA expressed in transgenic sugarcane tissue at the level found in this study, i.e., ≈0.9%, may contribute to significantly reducing population levels of *E. loftini* in the Lower Rio Grande Valley. Recent field studies showed a reduction in percent internodes bored by lepidopteran stalkborers (mostly *E. loftini*) in GNA-expressing transgenic sugarcane compared with near-isogenic sugarcane (J.C.L. et al., unpublished data). The nil to positive influence of GNA on *D. saccharalis* at the level tested in this study may be explained by the combined effect of relatively shorter larval feeding period and larger size of this species relative to *E. loftini.* Larvae of *D. saccharalis* feed for approximately one week less and weigh up to twice as much as larvae of *E. loftini* (M.S., unpublished data). Thus, the amount of GNA ingested per larval body weight in this study may have been insufficient to adversely affect larval growth and development, and may have complemented the diet's protein content. De Leo et al. (1998) reported high mortality and reduced larval weight in *Spodoptera littoralis* Boisd. that fed on tobacco plants expressing high levels of mustard trypsin Proteinase inhibitor-2 (MTI-2), whereas larvae fed leaves from plants expressing low levels of MTI-2 showed a net weight gain and a faster development compared with control larvae. It is unclear at present whether higher concentrations of GNA, as expressed in transgenic sugarcane tissue, may contribute to reducing populations of *D. saccharalis* in Lower Rio Grande Valley sugarcane because adverse effects were not recorded in greenhouse experiments using potted plants (J.C.L. et al., unpublished data). However, *D. saccharalis* is under biological control and currently not an economic pest of sugarcane in the Lower Rio Grande Valley (Legaspi et al. 1997, Meagher et al. 1998). Therefore, *D. saccharalis* is not a principal target of GNA-expressing sugarcane, and significant detrimental effects on this species are not indispensable in the Lower Rio Grande Valley.

Similar variability in the effects of GNA on life history parameters of lepidopteran pests has been reported in recent studies. GNA affected almost all growth and development parameters (e.g., survival, weight, pupation, emergence and fecundity) measured in the legume pod borer, *Maruca vitrata* (F.) (Machuka et al. 1999), whereas only a reduction in larval weight was observed in tomato moth, *Lacanobia oleracea* L. (Fitches et al. 1997). The results for *E. loftini* showing reductions in the net reproductive rate and intrinsic rate of increase for individuals fed GNA containing diet recorded in this study, coincide with previous findings demonstrating detrimental effects of GNA on insects.

Nontarget effects of transgenic insecticidal crops are increasingly being examined at different trophic levels, including potential effects on natural enemies (Hilbeck et al. 1998, Bell et al. 1999, Birch et al. 1999, Losey et al. 1999, Jesse and Obrycki 2000, Wraight et al. 2000). Recent studies showed variable impacts of GNA on natural enemies including reduced fertility, egg viability, and longevity in twospotted ladybeetle [*Adalia bipunctata* (L.)] that fed on aphids colonizing GNA-expressing plants, whereas variably lower fecundity and male longevity, and greater brood sizes were observed in the parasitoid *Eulophus pennicornis* (Nees) developing on hosts fed diet containing GNA (Bell et al. 1999, Birch et al. 1999). Another recent study showed that plant-derived protease inhibitors (soybean Kunitz inhibitor) consumed by herbivorous larvae are subsequently present in their hemolymph and thus readily transferable to parasitoids (Down et al. 2000). Because *D. saccharalis* is under highly effective biological control in the Lower Rio Grande Valley, it is important that any indirect effects of GNA

on natural enemies are identified before wide-scale deployment of GNA-expressing transgenic cultivars (Fuchs et al. 1979, Pfannenstiel and Meagher 1991, Meagher et al. 1998). Knowledge of any such effects would contribute additional information to the decision-making process concerning incorporation of GNA-based host plant resistance into existing pest management strategies in Lower Rio Grande Valley sugarcane. Biological control of *D. saccharalis* in the Lower Rio Grande Valley is attributed to a parasitoid, *Cotesia flavipes* (Cameron), and must not be compromised with wide-scale deployment of GNA-based resistance against *E. loftini.* A shorter larval period was observed in this study in *D. saccharalis* fed transgenic diet, and its potential effect on population dynamics of larval parasitoids, such as *C. flavipes,* remains unclear. In addition, the potential direct effects of GNA consumption by developing *C. flavipes* larvae also remain unclear. Similarly, the effects observed in *E. loftini* fed transgenic-diet may affect the population dynamics of parasitoid species shared with *D. saccharalis;* a recent study showed that six of seven common parasitoids of *D. saccharalis* are also parasitic on *E. loftini* (Meagher et al. 1998). Prior studies in other agroecosystems show that changes in host developmental rates can lead to differential levels of biological control by parasitoids (Luck and Podoler 1985, Luck et al. 1995, Devine et al. 2000). Other studies show that slight reductions in size (≈5%) of hosts reared on partially resistant host plants led to substantial reductions in size (≈34%), fecundity (≈26%), ratio of females (≈19%), and emergence rates (≈22%) of the parasitoid *Aphidius rhopalosiphi* De Stefani-Perez (van Emden 1995). However, in this study the effects of the transgenic diet on both *D. saccharalis* and *E. loftini* were variable between generations and it remains unclear how such variability may affect populations of these insects and their natural enemies in the field. Moreover, the transgenic diet as tested in this study had a GNA concentration equivalent to 50% of that expressed in transgenic sugarcane tissues. Thus, the effects observed on *D. saccharalis* and *E. loftini* in this study offer only a partial perspective on the potential effects that may occur under field conditions. Ongoing studies are focused on examining the effects on both *D. saccharalis* and *E. loftini,* and their principal parasitoids, of developing on diets (both plant tissue and artificial diet) containing concentrations of GNA higher than considered in this study (Sétamou et al. 2002).

## Acknowledgments

F. Moonan, Z. Yang (Texas Agricultural Experiment Station, Weslaco) were instrumental in developing the transgenic sugarcane. We thank R. Saldaña, S. Alvarez, M. Garcia, J. Huerta, R. Diaz, D. Alejandro, A. Cardoza, and M. V. Garza (all at TAES, Weslaco) for their technical assistance. C. Borgemeister (University of Hannover, Germany), T. X. Liu (TAES, Weslaco), and C. Rodríguez-Saona (USDA-ARS, Phoenix, AZ) reviewed the manuscript and provided numerous suggestions for its improvement. USDA-NRI-CGP Project #99-35316-7913, Hatch Projects #8595 and #8707, and Rio Grande Valley Sugar Growers, Incorporated, provided funding for this research.

## References Cited

**Allsopp, P. G., and T. K. McGhie. 1996.** Snowdrop lectin and wheatgerm lectins as antimetabolites for the control of sugarcane whitegrubs. Entomol. Exp. Appl. 80: 409–414.

**Bell, H. A., E. C. Fitches, R. E. Down, G. C. Marris, J. P. Edwards, J. A. Gatehouse, and A.M.R. Gatehouse. 1999.** The effect of snowdrop lectin (GNA) delivered via artificial diet and transgenic plants on *Eulophus pennicornis* (Hymenoptera: Eulophidae), a parasitoid of the tomato moth *Lacanobia oleracea* (Lepidoptera: Noctuidae). J. Insect Physiol. 45: 983–991.

**Birch, A.N.E., I. E. Geoghegan, M.E.N. Majerus, J. W. McNicol, C. Hackett, A.M.R. Gatehouse, and J. A. Gatehouse. 1999.** Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. Mol. Breeding 5: 75–83.

**Bradford, M. M. 1976.** A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

**De Leo, F., M. A. Bonadé-Bottino, L. R. Ceci, R. Gallerani, and L. Jouanin. 1998.** Opposite effects on *Spodoptera littoralis* larvae of high expression level of a trypsin proteinase inhibitor in transgenic plants. Plant Physiol. 118: 997–1004.

**Devine, G. J., D. J. Wright, and I. Denholm. 2000.** A parasitic wasp (*Eretmocerus mundus* Mercet) can exploit chemically induced delays in the development rates of its whitefly host (*Bemisi tabaci* Genn.). Biol. Control 19: 64–75.

**Down, R. E., L. Ford, S. D. Woodhouse, R.J.M. Raemaekers, B. Leitch, J. A. Gatehouse, and A.M.R. Gatehouse. 2000.** Snowdrop lectin (GNA) has no acute toxic effects on a beneficial insect predator, the twospotted ladybird (*Adalia bipunctata* L.). J. Insect Physiol. 46: 379–391.

**Fitches, E., A.M.R. Gatehouse, and J. A. Gatehouse. 1997.** Effects of snowdrop lectin (GNA) delivered via artificial diet and transgenic plants on the development of the tomato moth (*Lacanobia oleracea*) larvae in laboratory and glasshouse trials. J. Insect Physiol. 43: 727–739.

**Fuchs, T. W., F. R. Harding, and J. W. Smith, Jr. 1979.** Introduction and establishment of *Apanteles flavipes* [Hym.: Braconidae] on *Diatraea saccharalis* [Lep.: Pyralidae] in Texas. Entomophaga 24: 109–114.

**Gatehouse, A.M.R., G. Davidson, C. A. Newell, A. Merryweather, W.D.O. Hamilton, E. J. Burgess, R.J.C. Gilbert, and J. A. Gatehouse. 1997.** Transgenic potato plants with enhanced resistance to the tomato moth, *Lacanobia oleracea:* growth room trials. Mol. Breeding 3: 49–63.

**Hilbeck, A., M. Baumgartner, P. M. Fried, and F. Bigler. 1998.** Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27: 480–487.

**Hilder, V. A., A. M.R Gatehouse, S. E. Sheerman, R. F. Barker, and D. Boulter. 1987.** A novel mechanism of insect resistance engineered into tobacco. Nature (Lond.) 300: 160–163.

**Hulting, F. L., D. B. Orr, and J. J. Obrycki. 1990.** A computer program for calculation and statistical comparison of intrinsic rates of increase and associated life table parameters. Fla. Entomol. 73: 601–612.

**Irvine, J. E., and T. E. Mirkov. 1997.** The development of genetic transformation of sugarcane in Texas. Sugar J. 60: 25–29.

**Jesse, L.C.H., and J. J. Obrycki. 2000.** Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. Oecologia 125: 241–248.

**Kaku, H., and I. Goldstein. 1989.** Snowdrop lectin. Methods Enzymol. 179: 327–331.

**Legaspi, J. C., B. C. Legaspi, Jr., E. G. King, and R. Saldaña. 1997.** Mexican rice borer, *Eoreuma loftini* (Lepidoptera: Pyralidae) in the Lower Rio Grande Valley of Texas: its history and control. Subtrop. Plant Sci. 49: 53–59. et al. 1997.

**Leple, J. C., M. Bonade-Bottino, S. Augustin, G. Pilate, V. D. Le Tan, A. Deplanque, D. Cornu, and L. Jouanin. 1995.** Toxicity of to *Chrysomela tremulae* (Coleoptera: Chrysomelidae) of transgenic poplars expressing a cysteine proteinase inhibitor. Mol. Breeding 1: 319–328.

**Losey, J. E., L. S. Rayor, and M. E. Carter. 1999.** Transgenic pollen harms monarch larvae. Nature (Lond.) 399: 214.

**Luck, R. F., and H. Podoler. 1985.** Competitive exclusion of *Aphytis lingnanensis* by *A. melinus:* potential role of host size. Ecology 66: 904–913.

**Luck, R. F., M. J. Tauber, and C. A. Tauber. 1995.** The contributions of biological control to population and evolutionary ecology, pp. 25–45. *In* J. R. Nechols, L. A. Andres, J. W. Beardsley, R. D. Goeden, and C. G. Jackson (eds.), Biological control in the western United States, accomplishments and benefits of Regional Research Project W-84, 1964–1989. University of California, Division of Agriculture and Natural Resources, Oakland.

**Machuka, J., E.J.M. van Damme, W. J. Peumans, and L.E.N. Jackai. 1999.** Effect of plant lectins on larval development of the legume pod borer, *Maruca vitrata.* Entomol. Exp. Appl. 93: 179–187.

**Martinez, A. J., J. Bard, and T. A. Holler. 1988.** Mass rearing sugarcane borer and Mexican rice borer for production of parasites *Allorhogas pyralophagus* and *Rhaconotus rosilensis.* U.S. Dep. Agric.-APHIS-PPQ, APHIS 83–1.

**Meagher, R. L., Jr., J. E. Irvine, R. G. Breene, R. S. Pfannenstiel, and M. Gallo-Meagher. 1996.** Resistance mechanisms of sugarcane to Mexican Rice Borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 89: 536–543.

**Meagher, R. L., Jr., J. W. Smith, Jr., H. W. Browning, and R. R. Saldaña. 1998.** Sugarcane stemborers and their parasites in southern Texas. Environ. Entomol. 27: 759–766.

**Nutt, K. A., P. G. Allsopp, T. K. McGhie, K. M. Shepherd, P. A. Joyce, G. O. Taylor, R. B. McQualter, and G. R. Smith. 1999.** Transgenic sugarcane with increased resistance to canegrubs. Proc. Aust. Soc. Sugar Cane Technol. 21: 171–176.

**Pfannenstiel, R. S., and R. L. Meagher, Jr. 1991.** Sugarcane resistance to stalkborers (Lepidoptera: Pyralidae) in south Texas. Fla. Entomol. 74: 300–305.

**Powell, K. S., A.M.R. Gatehouse, V. A. Hilder, E.J.M. van Damme, W. J. Peumans, J. Boonjawat, K. Horsham, and J. A. Gatehouse. 1995.** Different antimetabolic effects of related lectins towards nymphal stages of *Nilaparvata lugens.* Entomol. Exp. Appl. 75: 61–65.

**Rao, K. V., K. S. Rathore, T. K. Hodges, X. Fu, E. Stoger, D. Sudhakar, S. Williams, P. Christou, M. Bharathi, and D. P. Brown. 1998.** Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown planthopper. Plant J. 15: 469–477.

**Rodríguez-del-Bosque, L. A., J. W. Smith, Jr., and H. W. Browning. 1989.** Development and life-tables of *Diatraea lineolata* (Lepidoptera: Pyralidae) at constant temperatures. Ann. Entomol. Soc. Am. 82: 450–459.

**SAS Institute. 1996.** SAS user's guide, statistics. SAS Institute, Cary, NC.

**Schuler, T. H., G. M. Poppy, B. R. Kerry, and I. Denholm. 1998.** Insect resistant transgenic plants. TIBTECH 16: 168–175.

**Sétamou, M., F. Schulthess, N. A. Bosque-Pérez, H.-M. Poehling, and C. Borgemeister. 1999.** Bionomics of *Mussidia nigrivenella* (Lepidoptera: Pyralidae) on three host plants. Bull. Entomol. Res. 89: 465–471.

**Sétamou, M., J. S. Bernal, J. C. Legaspi, B. Legaspi, and T. E. Mirkov. 2002.** Effects of snowdrop lectin (GNA) expressed in transgenic sugarcane on fitness of *Cotesia flavipes* (Cameron), a parasitoid of the non-target pest *Diatraea saccharalis* F. Ann. Entomol. Soc. Am. (in press).

**Sokal, R. R., and J. F. Rohlf. 1995.** Biometry, 3rd ed. Freeman, New York.

**Stoger, E., S. Williams, P. Christou, R. E. Down, and J. A. Gatehouse. 1999.** Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphids *Sitobion avenae.* Mol. Breeding 5: 65–73.

**Van Damme, E.J.M., W. J. Peumans, A. Barre, and P. Rougé. 1998.** Plant lectins: a composite of several distinct families of structure and evolutionary related proteins with diverse biological roles. Crit. Rev. Plant Sci. 17: 575–692.

**Van Emden, H. F. 1995.** Host plant-aphidophaga interactions. Agric. Ecosyst. Environ. 52: 3–11.

**Wraight, C. L., A. R. Zangerl, M. J. Carroll, and M. R. Berenbaum. 2000.** Absence of toxicity of Bacillus thuringiensis pollen to black swallowtails under field conditions. Proc. Natl. Acad. Sci. U.S.A. 97: 7700–7703.

**Zar, J. H. 1996.** Biostatistical analysis. Prentice-Hall, Upper Saddle River, NJ.

*Received for publication 5 June 2001; accepted 15 November 2001.*